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LETTER

Applicants call Examiner's attention to the attached reference (Liang et al., Ionization enhancement in atmospheric pressure chemical ionization and suppression in electrospray ionization between target drugs and stable-isotope-labeled internal standards in quantitative liquid chromatography/tandem mass spectrometry, Rapid Communications in Mass Spectrometry, 2003, 17: 2815-2821), which is placed in the file for the record.

Respectfully submitted,

Date: June 8, 2010

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Ionization enhancement in atmospheric pressure chemical ionization and suppression in electrospray ionization between target drugs and stable-isotope-labeled internal standards in quantitative liquid chromatography/tandem mass spectrometry†

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The phenomena of ionization suppression in electrospray ionization (ESI) and enhancement in atmospheric pressure chemical ionization (APCI) were investigated in selected-ion monitoring and selected-reaction monitoring modes for nine drugs and their corresponding stable-isotope-labeled internal standards (IS). The results showed that all investigated target drugs and their co-eluting isotope-labeled IS suppress each other's ionization responses in ESI. The factors affecting the extent of suppression in ESI were investigated, including structures and concentrations of drugs, matrix effects, and flow rate. In contrast to the ESI results, APCI caused seven of the nine investigated target drugs and their co-eluting isotope-labeled IS to enhance each other's ionization responses. The mutual ionization suppression or enhancement between drugs and their isotope-labeled IS could possibly influence assay sensitivity, reproducibility, accuracy and linearity in quantitative liquid chromatography/mass spectrometry (LC/MS) and liquid chromatography/tandem mass spectrometry (LC/MS/MS). However, calibration curves were linear if an appropriate IS concentration was selected for a desired calibration range to keep the response factors constant.

Stable-isotope-labeled analogs are commonly used as internal standards (IS) in quantitative gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/mass spectrometry (LC/MS).[1–5] The stable-isotope-labeled analogs are chemically and structurally the same as their target drugs but differ in molecular mass. The primary reason for utilizing a stable-isotope-labeled analog is to normalize the response of a given target drug to the response of its isotopic analog and thus compensate for variations in injection, sample preparation, instrumental parameters and matrix effects.[6–11] Previous reports have noted that, when constructing calibration curves with electrospray ionization (ESI), the peak areas of the co-eluting labeled IS decreases with increasing drug concentrations.[12,13] There are also some reports on ionization suppression in atmospheric pressure chemical ionization (APCI).[14,15] We have also noticed the above behavior when using ESI,[16] but with APCI we found that the peak areas of stable-isotope-labeled IS increased when drug concentrations increased.

To further explore these phenomena and their possible influence on assay sensitivity, reproducibility, accuracy and linearity, we investigated the effects of ionization suppression in ESI and enhancement in APCI between nine drugs and their corresponding stable-isotope-labeled internal standards.

EXPERIMENTAL

(R)- and (S)-Methadone and methadone-D_9 were purchased from Cerilliant (Austin, TX, USA). Fructose, sorbitol, fructose-$^{13}C_6$, sorbitol-$^{13}C_6$, fructose-D_2 and sorbitol-D_2 were purchased from Omicron Biochemicals, Inc. The other six drugs and their corresponding isotope-labeled internal standards are proprietary.

All chemicals were of analytical-reagent grade: hexane, methanol, isopropyl alcohol (IPA) and acetonitrile from EM Science (Gibbstown, NJ, USA); ammonium acetate and sodium bicarbonate from VWR Scientific Products (West Chester, PA, USA).

Chromatography was performed using a Shimadzu SCL-10A controller with CL-10AD pump and CTO-10A column

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oven (Shimadzu Scientific Instruments, Inc., Columbia, MD, USA). The autosampler was a PE series 200 (Perkin Elmer, Hopkinton, MA, USA). The API 3000 and 4000 tandem mass spectrometers were from MDS Sciex (Concord, Ontario, Canada).

The target drugs II–VII and their labeled IS were chromatographed using a Synergi Hydro-RP or Prodigy phenyl-3 column (30 × 2 mm, 5 μm particle size) with a mobile phase containing MeCN/5 mM ammonium acetate or 0.1% formic acid at a flow rate of 300 μL/min and an oven temperature of 40°C. (R)- and (S)-Methadone were chromatographed using a Chiral-AGP column (50 × 2 mm, 5 μm particle size) with a mobile phase containing isopropyl alcohol in ammonium acetate at a flow rate of 400 μL/min and at an oven temperature of 45°C. Fructose, sorbitol, fructose-$^{13}C_6$, sorbitol-$^{13}C_6$, fructose-D_2 and sorbitol-D_2 were chromatographed on a CapCell Pak 5 μ NH_2 column (UG-80A, 150 × 4.6 mm) with a mobile phase containing MeCN/MeOH/water.

The API 3000 and 4000 triple-quadrupole mass spectrometers were operated in either selected-ion monitoring (SIM) or selected-reaction monitoring (SRM) mode under optimized conditions for detection of positive or negative ions of drugs and IS formed by TurboIonSpray™ ionization (ESI) or heated nebulizer ionization (APCI). Drugs VIII and IX were studied using the API 4000, and the other drugs using the API 3000. Drugs V, VIII and IX were studied in negative ion mode and other drugs in positive ion mode.

The isotope-labeled IS and their target drugs were prepared in mobile phase. To explore the effects of matrix on the extent of suppression in ESI, 5000 ng/mL of fructose-$^{13}C_6$, 2500 ng/mL of sorbitol-$^{13}C_6$, fructose-D_2 and sorbitol-D_2 were spiked into 200 μL of human red blood cells and water in 10 replicates, and extracted with protein precipitation using acetonitrile.

The effect of flow rate on the extent of response suppression was studied by the injection of 20 μL of 1000 ng/mL drug II and the post-column infusion of drug II-13C6 at the rate of 20 μL/min while changing the chromatographic flow rate from 0.1 to 0.8 mL/min.

The experiments to investigate ionization suppression and enhancement were performed using a post-column infusion system in which a constant flow of a drug (or its IS) was infused post-column into the MS detector and the IS (or the drug) injected by an autosampler onto the analytical column.[17,18] The purpose of the post-column infusion with a drug (or its IS) is to raise the background level so that the suppression by the IS (or the drug) will show as negative peaks and the enhancement will show as positive peaks. The extent of ionization suppression or enhancement was also measured by comparing the peak areas of drug or IS from solutions containing only drug or IS or both of them.

The chromatographic peaks for the target drugs and their corresponding stable-isotope-labeled IS were integrated using Analyst software (version 1.2) with a smoothing factor of one. Quantitation was based on linear regression analysis of calibration curves (weighted 1/x) using the analyte to IS area ratio vs. target concentration utilizing Watson® DMLIMS software (version 6.1.1.04).

RESULTS AND DISCUSSION

Table 1 lists information on the nine investigated drugs. These included basic, acidic and neutral compounds, and their deuterium- or ^{13}C-labeled internal standards.

Ionization suppression in ESI

Ionization suppression between target drugs and their stable isotope-labeled IS in SIM and SRM modes

The results showed that, when using ESI, all target drugs suppressed the ionization responses of their co-eluting labeled IS in both SIM and SRM modes, and likewise the labeled IS suppressed the ionization responses of the corresponding target drugs. Figure 1 shows a representative example of the results. This suppression can be understood in terms of Enke's model of ESI ion generation,[19,20] which involves a result of the competition among ions for the limited number of excess charge sites on the generated droplet during ESI. This model predicts the response curves of singly charged ionic analytes as a function of the concentration of electrolyte and other analytes. The extents of suppression of signals from the nine target drugs by their corresponding stable-isotope-labeled IS are listed in Table 1.

Table 1. Nine investigated target drugs and their corresponding isotope-labeled internal standards (IS)

Analytes	Analytes labeled IS					
	$[M+H]^+$ (m/z)	IS	$[M+H]^+$ (m/z)	Isotopic contribution*	Structure type	Extent of suppression** %
I (R)-, (S)-methadone	310	D_3	313	0.2%	Tertiary amine	72
II	409	$^{13}C_6$	415	<0.2%	Primary amine	68
III	478	$^{13}C_6$, D_2	482	<0.2%	Secondary amine	84
IV	506	$^{13}C_6$, D_1	510	<0.2%	Tertiary amine	82
V	474	D_4	478	0.2%	Acidic	80
VI	249	D_3	252	0.5%	Neutral	62
VII	265	D_3	268	0.5%	Neutral	64
VIII sorbitol	181	$^{13}C_6$	187	<0.2%	Neutral	35
IX fructose	179	$^{13}C_6$	185	<0.2%	Neutral	40

*Isotopic contribution indicates contribution of naturally occurring isotopic abundance of drugs to their corresponding isotope-labeled IS response assuming equal concentrations of the drug and the IS.

**Extent of suppression was calculated as: 100 × [(intensity of signal from drug by post-column infusion – signal intensity of drug suppressed due to the on-column injection of their IS)/intensity of signal from drug by post-column infusion]. Concentrations of drugs and their IS: 10 μg/mL, injection volume: 20 μL; infusion rate: 20 μL/min.

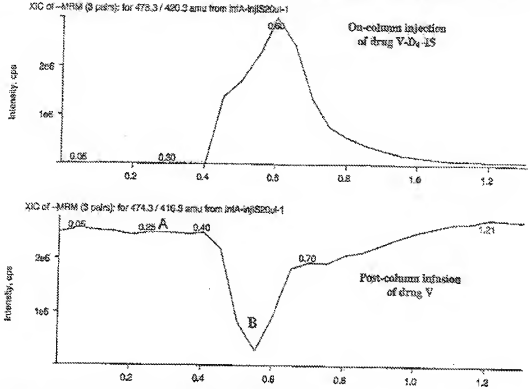

Figure 1. ESI response of drug V suppressed ~80% by its D_4-IS during the elution window of D_4-IS. (A) Intensity of D_0 by post-column infusion and (B) intensity of D_0 suppressed due to the on-column injection of D_4. Using a post-column infusion system, drug V was infused post-column and its D_4 analog was injected on-column. For other conditions, see Experimental section. Concentrations of drug V and its D_4 analog were 10 μg/mL; injection volume: 20 μL; infusion rate: 20 μL/min. The extent of suppression % was calculated as: 100 × [(A − B)/A].

Factors affecting the extent of suppression

Concentrations of investigated drugs and IS. Figure 2 illustrates the mutual ionization suppression between methadone

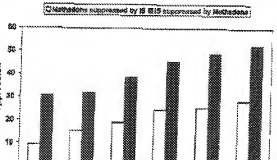

Figure 2. ESI suppression between methadone and methadone-D_9-IS. The white bars indicate the extent of suppression of D_0 ionization by D_9 by comparing the LC/MS/MS peak areas of D_0 from the solutions containing only D_0 with those containing both D_0 and D_9. The black bars indicate the extent of suppression of D_9 ionization by D_0 by comparing the peak areas of D_9 from the solutions containing only D_9 with those containing both of them. Concentrations of D_0: 50–10 000 ng/mL; concentrations of D_9: 25–5000 ng/mL. For other conditions, see Experimental section.

(from 50–10 000 ng/mL) and its D_9-IS (25–5000 ng/mL). The results demonstrate that, as the concentration of the D_9-IS increased, the greater the suppression of methadone D_0 was caused by its D_9-IS, and vice versa. For example, at the concentrations 50/25 ng/mL of D_0/D_9, the suppression was approximately 10%/30%, respectively. At the 5000/2500 ng/mL concentration level the suppression was 25%/50%. The extent of suppression in each drug-IS pair was concentration-dependent in a nonlinear fashion.

Hydrophobicity of investigated drugs. The extent of suppression was related to the structures of the investigated drugs (Table 1). Under optimized LC/MS/MS conditions, the extent of suppression for each investigated drug by its IS was different when the concentration, injection volume, and infusion rate of drugs or IS were exactly the same. The hydrophobicity was determined based on organic vs. aqueous partitioning. Generally, the level of suppression was correlated with the hydrophobicity of the compounds; the more hydrophobic the compound, the lower the level of suppression. However, fructose and sorbitol, both hydrophilic compounds, did not comply with this general finding. Only 35–40% of sorbitol or fructose response was suppressed while ~60% of the signals of two hydrophobic drugs, VI and VII, were suppressed.

Matrix effects. Fructose-$^{13}C_6$ and sorbitol-$^{13}C_6$ and/or fructose-D_2 and sorbitol-D_2 were spiked into human red blood

Table 2. Ionization suppression in ESI between fructose-$^{13}C_6$ (sorbitol-$^{13}C_6$) and fructose-D_7 (sorbitol-D_7) extracted from human red blood cells (RBCs) and water, respectively

Peak areas of fructose-, sorbitol-$^{13}C_6$ and D_7 spiked only with $^{13}C_6$ or D_7 in water				
(n = 10)	fructose-$^{13}C_6$	fructose-D_7	sorbitol-$^{13}C_6$	sorbitol-D_7
AVE	7869000	3301000	258 62500	311 45500
Std Dev	377395	110 4300	713470	144 6033
CV%	0.48	3.3	2.7	4.6
Peak areas of fructose-, sorbitol-$^{13}C_6$ and D_7 spiked with both $^{13}C_6$ and D_7 in water				
(n = 10)	fructose-$^{13}C_6$	fructose-D_7	sorbitol-$^{13}C_6$	sorbitol-D_7
Ave	6673600	2183800	224 85000	278 81500
Std Dev	146 3711	555 785	274 857	184 554
CV%	2.1	2.5	1.2	0.6
Suppression %	15.1	33.8	13.0	10.4
Peak areas of fructose-, sorbitol-$^{13}C_6$ and D_7 spiked only with $^{13}C_6$ or D_7 in human RBCs				
(n = 10)	fructose-$^{13}C_6$	fructose-D_7	sorbitol-$^{13}C_6$	sorbitol-D_7
AVE	489 84500	608 6050	165 78500	834 0900
Std Dev	109 15607	244 6660	493 3484	267 8833
CV%	22.2	40.2	29.7	32.0
Peak areas of fructose-, sorbitol-$^{13}C_6$ and D_7 spiked with both $^{13}C_6$ and D_7 in human RBCs				
(n = 10)	fructose-$^{13}C_6$	fructose-D_7	sorbitol-$^{13}C_6$	sorbitol-D_7
AVE	131 68900	297 9030	443 5550	493 3700
Std Dev	150 5430	362 250	414 293	391 595
CV%	11.4	12.1	9.343304	7.937161
Suppression %	73.1	51.0	73.2	40.8

The suppression (%) of fructose- and sorbitol-$^{13}C_6$ by their D_7 (or D_2 by $^{13}C_6$) was determined by comparing the peak areas of $^{13}C_6$ (or D_7) from solutions containing only $^{13}C_6$ (or D_7), or both $^{13}C_6$ and D_7. Concentration of fructose-$^{13}C_6$: 5000 pg/mL; concentration of sorbitol-$^{13}C_6$ or D_7: 2500 ng/mL; injection volume: 10 μL.

cells and water, respectively, and extracted with the exact same procedures. As shown in Table 2 there were significant matrix effects; there were significant differences in signal suppression for fructose-$^{13}C_6$ and sorbitol-$^{13}C_6$ by their D_7 (or D_2 by $^{13}C_6$) in human red blood cells compared with water. The signals were suppressed more in human red blood cells compared with those in water. This result can also be understood in terms of Enke's model of ESI ion generation,[19,20] a result of the competition among ions for the limited number of excess charge sites on the generated droplet during ESI.

Flow rate. Generally, the higher the flow rate, the higher the level of suppression. Figure 3 shows a representative result. The signal suppression of analytes tended to be lower at lower flow rates. This can also be explained by Enke's model in which the sum of the excess charge concentration at the surface is inversely proportional to flow rate.[19,20]

Effect of ionization suppression on assay sensitivity, reproducibility, accuracy and linearity

Table 3 shows the influence of (R)-methadone-D_3 (IS) concentrations on the sensitivity, reproducibility, accuracy and linearity for (R)-methadone. The IS concentrations influenced the detection limit and the lower limit of quantitation (LLOQ) of the assay. As an example of this effect, without addition of the IS the response for 1 ng/mL of (R)-methadone was observed as a peak with signal-to-noise (S/N) ratio > 10, but in the presence of 200 ng/mL of IS the response for 1 ng/mL of (R)-methadone was totally suppressed by the IS. When the IS concentration was increased to 10 000 ng/mL, the responses for up to 10 ng/mL of (R)-methadone were totally suppressed and the response of 25 ng/mL of (R)-methadone was suppressed up to 95%.

The IS concentration also influenced the reproducibility, response factors, accuracy and linearity of the assay because the mutual suppression was concentration-dependent. The results appeared to be contrary to those of a previous report,[12] in which Sojo *et al.* found that the mutual suppression did not have an effect on the calibration curve and quantitation of target drugs. Based on the present results, the reproducibility was better for the IS responses and worse for the response factors and accuracy of the unlabeled drug; also the linear range was narrower with different slope and correlation of determination in the calibration series in the presence of 10 000 ng/mL of IS than in the presence of 200 ng/mL of IS. In the presence of 10 000 ng/mL of IS, the

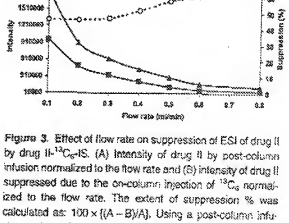

Figure 3. Effect of flow rate on suppression of ESI of drug II by drug II-$^{13}C_6$-IS. (A) Intensity of drug II by post-column infusion normalized to the flow rate and (B) intensity of drug II suppressed due to the on-column injection of $^{13}C_6$ normalized to the flow rate. The extent of suppression % was calculated as: 100 × [(A − B)/A]. Using a post-column infusion system, drug II was infused post-column and its $^{13}C_6$ was injected on-column. Concentrations of drug II and its $^{13}C_6$: 1 μg/mL; injection volume: 20 μL; infusion rate: 20 μL/min. For other conditions, see Experimental section.

Table 3. Influence of (R)-methadone-D_9 internal standard (IS) concentrations on sensitivity, precision, accuracy and linearity of (R)-methadone in calibration curves (n = 4)

Concentration of (R)-methadone (ng/mL)	IS peak area	Response factor*	Accuracy	(R)-Methadone peak area	Suppression %**
Without addition of IS					
1		2001	152.0	2001.1	
5		2406	143.2	12 028	
25		2573	230.9	64 331	
50		1957	180.5	97 827	
100		1830	173.5	182 960	
500		1344	133.4	671 830	
2000		966	104.6	193 1300	
5000		527	75.7	263 3700	
8500		541	85.6	459 5100	
10000		527	N/A	527 4000	
AVE		1295	140.6		
STD DEV		763	72.4		
CV%		58.9	51.5		
With addition of 200 ng/mL of IS					
1	192 880	0.00000	N/A	N/A	N/A
5	180 610	0.01079	112.1	5812	26.7
25	165 640	0.01086	92.5	52 365	18.6
50	165 520	0.01068	88.5	96 432	1.4
100	163 370	0 01059	86.6	175 360	4.2
500	113 550	0.01107	89.4	628 500	6.4
2000	71 356	0.01236	103.7	183 5300	5.0
5000	55 170	0.01255	101.1	249 8700	5.1
8500	41 192	0.01310	105.6	458 8000	0.2
10000	35 827	0.01275	94.7	594 4400	0.6
AVE	101 953	0.01168	95.3		
STD DEV	56 921	0.00103	7.3		
CV%	55.8	8.8	7.7		
R^{2}***		0.999 from 5 to 10 000 ng/mL			
With addition of 10 000 ng/mL of IS					
1	257 4600	N/A	N/A	N/A	N/A
5	214 3500	N/A	N/A	N/A	N/A
25	211 9600	0.00007	181.0	4479	93.0
50	224 9300	0.00012	122.1	13 428	86.3
100	218 8100	0.00016	102.4	35 171	80.8
500	229 9800	0.00021	93.7	271 930	59.5
2000	193 0100	0.00024	99.1	109 3200	43.4
5000	146 6000	0.00023	96.4	173 7500	34.0
8500	170 4800	0.00025	102.7	362 1000	21.2
10000	218 1100	0.00019	89.0	419 0100	20.6
AVE	201 9850	0.00019	112.0		
STD DEV	269 726	0.00006	29.2		
CV%	14.3	34.9	26.0		
R^{2}***		0.954 from 25 to 10 000 ng/mL			

* Response factor: response factor refers to the peak area ratio (drug/internal standard) vs. drug concentration.

** Suppression %: %{[(R)-methadone peak areas obtained without addition of IS] – [(R)-methadone peak areas obtained with addition of IS)]/[(R)-methadone obtained without addition of IS].

*** R^2, correlation of determination.

responses of (R)-methadone at low concentrations were suppressed to a greater extent (up to 93%) than at higher concentrations (20%). In the presence of 200 ng/mL of IS, the extent of suppression of the drug responses varied from 26 to 0.3%.

Therefore, it is important to select an appropriate IS concentration for a desired calibration range to keep calibration curves linear. Generally, IS concentration should not be too high. An appropriate IS concentration depends on the investigated drugs and other experimental parameters and thus should be determined by experiment. For the methadone assay, the appropriate IS concentration was found to be 200 ng/mL for the desired linear range from 5–10 000 ng/mL, with $R^2 = 0.999$ and overall %CV of response factors = 8.8%.

On the other hand, the extent of suppression of the IS responses depended on the drug concentrations. The suppression of the IS response by its target drug caused the apparent poor reproducibility of the IS response. Figure 4 shows that the peak areas of co-eluting labeled (R)- and (S)-methadone-D_9 decreased as the concentration of (R)- and (S)-methadone increased in the calibration series. This behavior was also IS concentration-dependent and was not significant in the presence of 10 000 ng/mL of IS (Table 3).

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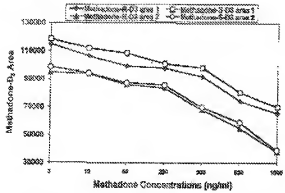

Figure 4. Electrospray peak areas of co-eluting labeled (R)- and (S)-methadone-D_6-IS decreasing with increasing (R)- and (S)-methadone concentrations in a calibration series. Areas 1 and 2 are from results obtained on different days. For conditions, see Experimental section.

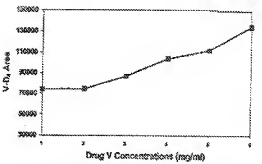

Figure 6. LC/APCI-MS/MS peak areas of D_4-IS increase with increasing drug V concentration. For conditions, see Experimental section.

Ionization enhancement in APCI

Ionization enhancement between target drugs and their stable-isotope-labeled IS in SIM and SRM modes

In contrast to the ESI results, seven out of the nine investigated target drugs enhanced the ionization responses of the corresponding labeled IS in both SIM and SRM modes, and likewise the labeled IS enhanced the responses of the target drugs with APCI. The responses of drugs I–VII were enhanced 2–7 times by their corresponding IS. Figure 5 shows that the response of drug V was enhanced ~7 times by its D_4 analog. However, the ionization responses of 10 μg/mL of fructose and sorbitol by post-column infusion were not enhanced by on-column injection of 10 μg/mL of fructose-$^{13}C_6$ or sorbitol-$^{13}C_6$, but rather were suppressed

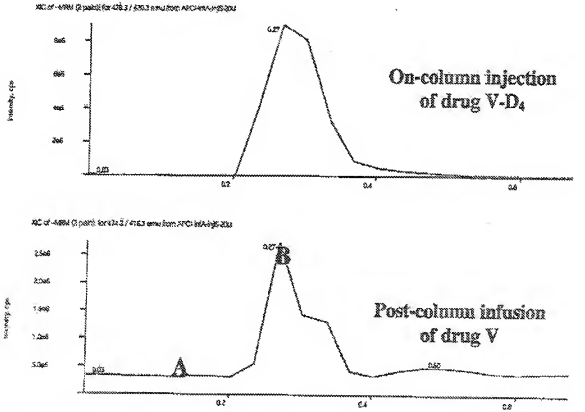

Figure 5. APCI response of drug V enhanced ~7 times by its D_4-IS during the retention time window of the D_4-IS. (A) Intensity of D_0 by post-column infusion and (B) intensity of D_0 enhanced due to the on-column injection of D_4. Using a post-column infusion system, drug V was infused post-column and its D_4-IS was injected on-column. The extent of enhancement was calculated as ((B – A)/A). Concentrations of drug V and its D_4-IS: 10 μg/mL; injection volume: 20 μL; infusion rate: 20 μL/min. For other conditions, see Experimental section.

Table 4. Precision of IS peak area ($V\text{-}D_4$), response factor and accuracy of drug V in calibration curves (n = 2)

Concentration of drug V (mg/mL)	IS peak area	Response factor	Accuracy
0.400	74 056	2.81	99
0.800	74 451	3.08	105
2.00	86 968	3.03	104
4.00	103 870	2.87	98
6.00	112 246	2.76	95
7.20	134 690	3.07	105
AVE	97 725	2.93	101
STDV	23 810	0.15	4.3
CV%	24.3	5.4	4.3

by fructose-$^{13}C_6$ or sorbitol-$^{13}C_6$ when their concentrations were increased to 50 μg/mL.[14,15] Explanations for these results are still being explored.

Extent of enhancement and concentrations of investigated drugs

In some cases, the enhancement of IS signals by their corresponding drugs was concentration-dependent, and the peak areas for the IS increased with increasing drug concentrations in calibration curves. Figure 6 shows that the peak areas of the co-eluting $V\text{-}D_8$ increased with increasing drug V concentrations. However, this behavior was not significant for five of the nine investigated target drug-IS pairs.

Effect of ionization enhancement on assay sensitivity, reproducibility, accuracy and linearity

Ionization enhancement of drugs by their stable-isotope-labeled IS in APCI could possibly improve the detection limits and the LLOQ of the assay for some drugs. The increase of IS responses with increasing drug concentrations in the calibration series resulted in poor apparent reproducibility for the IS signal. However, calibration curves were linear if an appropriate internal standard concentration was selected for a desired calibration range to keep the response factor constant (Table 4).

Effect of natural isotopic contribution from drugs on enhancement of IS

The contributions of naturally occurring isotopic abundance of drugs to the signals for their corresponding isotope-labeled IS are shown in Table 1. The degree of enhancement of isotope-labeled IS was substantially greater than could be accounted for by the naturally occurring isotopic abundances of their target drugs.

Effect of purity of IS on enhancement of drugs

In the present study, the isotopic purities of all isotope-labeled IS used were over 99%. Therefore, the presence of any significant D_0-IS impurities was ruled out.

CONCLUSIONS

Ionization enhancement under APCI conditions between target drugs and co-eluting isotope-labeled IS was investigated in quantitative LC/MS and LC/MS/MS for the first time. In APCI, seven out of nine investigated target drugs and their co-eluting isotope-labeled IS were found to enhance each other's ionization responses. In ESI, all investigated target drugs and their co-eluting isotope-labeled IS were found to suppress each other's ionization responses. The mutual ionization enhancement and suppression between drugs and their isotope-labeled IS can influence assay sensitivity, reproducibility, accuracy and linearity. Linear calibration curves can be maintained if an appropriate IS concentration is selected for a desired calibration range to keep the response factor constant.

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